RO5503781
BRIEFING PACKAGE

PEDIATRIC ONCOLOGY SUBCOMMITTEE OF THE ONCOLOGIC DRUGS ADVISORY COMMITTEE MEETING

HOFFMANN-LA ROCHE INC.

NOVEMBER 2014

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AHD	antecedent hematological disorders
ALL	acute lymphoblastic leukemia
AML	acute myeloid leukemia
AUC	area under the concentration-time curve
AUROC	area under a receiver operator characteristic
BFM	Berlin-Frankfurt-Münster
CNS	central nervous system
CR	complete remission
CRi	complete remission with incomplete recovery of peripheral counts
DLT	dose-limiting toxicity
EFS	event-free survival
EMA	European Medicines Agency
FDA	U.S. Food and Drug Administration
HI	hematological improvements
K_D	dissociation constant
MDM2	murine double minute 2
MIC-1	macrophage inhibitory cytokine-1
MLFS	morphological leukemia-free state
MTD	maximum-tolerated dose
ODAC	Oncologic Drugs Advisory Committee
nutlin	Nutley inhibitor
p53	tumor protein p53 (protein)
PDCO	Paediatric Committee (EMA)
PIP	Paediatric Investigation Plan
PK	pharmacokinetic
PPTP	Pediatric Preclinical Testing Program
PR	partial response
QTc	corrected QT interval
QTcF	Fridericia's corrected QT interval
RP2D	recommended Phase 2 dose
SEER	Surveillance Epidemiology and End Results
t-AML	therapy-related AML
T/C	tumor/control
TGI	tumor growth inhibition
TP53	tumor protein p53 (gene)

1. EXECUTIVE SUMMARY

While the overall survival rates for certain pediatric cancers have improved over the past 40 years, survival rates greatly decrease when the cancers return, and there remains a significant unmet need for new treatment options.

The Sponsor, Hoffmann-La Roche (Roche) is seeking advice from the Pediatric Oncology Subcommittee of the Oncologic Drugs Advisory Committee (ODAC) regarding how to manage the challenges in studying RO5503781 in pediatric cancers.

p53 is a protein that regulates the cell cycle and suppresses tumor growth. Murine double minute 2 (MDM2) is a protein that blocks the tumor-suppressing activity of p53, leading to the survival and proliferation of cancer cells. RO5503781 is an investigational, oral medicine that is designed to disrupt the interaction between p53 and MDM2 to help restore activity that stops tumor growth and promotes apoptosis (programmed cancer cell death).

Unlike many adult cancers, several types of pediatric cancer have a high prevalence of tumors that are p53 wild-type, which may increase the likelihood of response from an MDM2 antagonist. Nonclinical data provide a strong rationale for studying RO5503781, and an international collaborative research consortium of investigators and Roche has been established to continue developing supportive nonclinical data for relevant pediatric cancer types.

RO5503781 is not approved for marketing in the United States, the European Union or any other region. Currently, Roche's clinical development program of RO5503781 is primarily focused on adult cancers in relapsed or refractory disease to inform future studies in pediatric cancers.

More than 95% of patients with pediatric leukemias such as acute lymphocytic leukemia (ALL) and acute myeloid leukemia (AML) will achieve complete remission after initial therapy with a chemotherapy regimen. However, a significant number of patients relapse (17.5% for ALL and 35% for AML). For pediatric patients with solid tumors such as neuroblastoma, osteosarcoma, Ewing's sarcoma and rhabdomyosarcoma, most of those whose disease relapses after initial treatment do not survive.

With these survival rates, there has been long standing interest in the development of RO5503781 for pediatric cancers. However, several challenges will need to be addressed:

- While there is a high prevalence of p53 wild-type tumors in several pediatric cancers, patients with some forms of mutated p53 may respond to therapy making it difficult to determine which patients are most likely to benefit from RO5503781 based on mutational status alone.

- Substantial variability in exposure of RO5503781 observed in adult patients may be problematic in pediatric patients who have a range of ages and body weights.
- The adult tablet formulation is not feasible for younger patients who are unable to swallow pills; however, RO5503781 is poorly soluble and in its amorphous state sensitive to moisture, which limits the possibility of a liquid formulation. Therefore, other age-appropriate formulations must be evaluated.

The following briefing document describes the mechanism of action of RO5503781 and data from nonclinical, clinical pharmacology, and early stage clinical studies in adult patients. It also provides an overview of the nonclinical pediatric studies and planned development in pediatric cancers driven by efficacy observed in adults.

2. DESCRIPTION OF THE MOLECULE

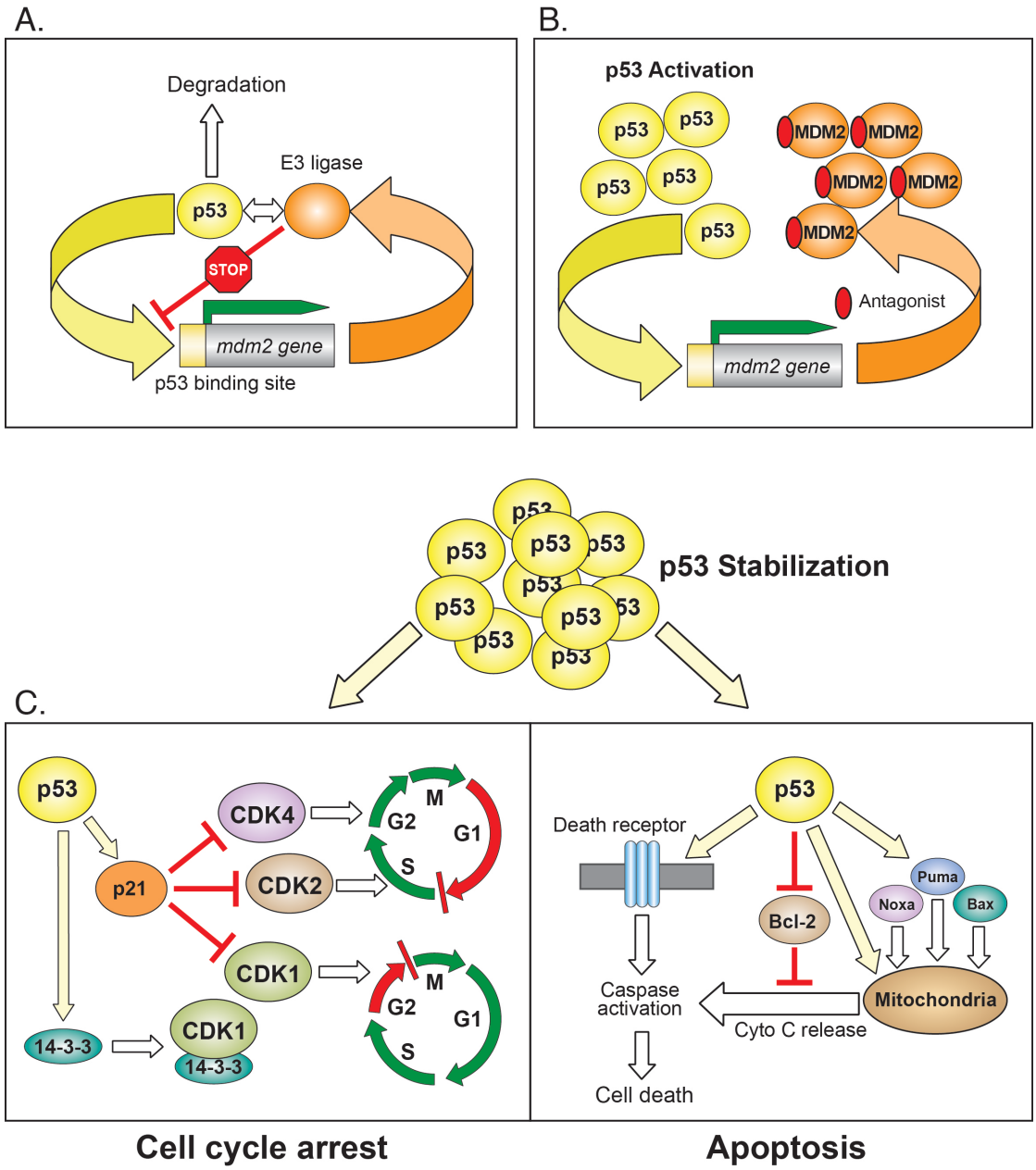
2.1 MECHANISM OF ACTION

MDM2 is a well-characterized oncogene and negative regulator of p53 (*TP53*), a key guardian of genomic integrity. The p53 tumor suppressor protein plays a prominent role in cell growth arrest and apoptosis, and its loss in cancer cells has critical implications for tumorigenesis (Levine 1997; Vogelstein et al. 2000; Zilfou and Lowe 2009). The anti-p53 effects of MDM2 occur through direct binding and inhibition of the transcriptional activation domain of p53, and by targeting p53 for ubiquitination and degradation (Momand et al. 1992; Kubbutat et al. 1997; Fahraeus and Olivares-Illana 2013) (Figure 1). In cancer cells, the normal functions of p53 are often inhibited, either by direct mutation of *TP53* or by dysregulation of the MDM2-p53 regulatory pathway. For example, overexpression of *MDM2* in a variety of cancers is a mechanism by which p53 functional activity may be inhibited, contributing to the survival and proliferation of the cancer. The importance of this pathway is evidenced by the embryonic lethality of *MDM2*-null mice and their rescue by knocking out *TP53* (Jones et al. 1995; Montes de Oca Luna et al. 1995). Activation of the normal cell growth arrest and apoptotic functions of p53 by blocking the MDM2-p53 interaction offers a promising therapeutic approach for cancer (Vassilev 2007; Brown et al. 2009; Cheok et al. 2011).

Genetic and biochemical studies have mapped the p53-MDM2 binding sites to the N-terminal domain of MDM2 and three critical amino acid residues in the transactivation domain of p53. The crystal structure of a p53-derived peptide bound to the p53 binding domain of MDM2 reveals a relatively deep cavity on the surface of MDM2 (Kussie et al. 1996). A class of small-molecule compounds has been identified as potent and selective inhibitors of the p53-MDM2 interaction (Vassilev et al. 2004). These MDM2 antagonists, termed nutlins (Nutley inhibitors), interact specifically with the p53-binding pocket of MDM2, and therefore free p53 from negative control. Targeting cancer cells that express wild-type p53 with nutlins stabilizes p53 and activates the p53 pathway, leading to expression of p53 target genes, cell cycle arrest, and apoptosis (Tovar et al. 2006; Vassilev 2007).

Nutlins have demonstrated significant activity in vivo with limited toxicity (Vassilev et al. 2004). As the first non-DNA–damaging (non-genotoxic) p53 activators to be developed as therapeutic agents, the mechanism of action of nutlins may be particularly relevant for pediatric indications.

Figure 1 Regulation of p53 Stability and Activity by MDM2



BAX = BCL-2-associated X protein; BCL-2 = B cell lymphoma 2 protein; CDKs = cyclin-dependent kinases; Cyto C = cytochrome C; G1 = Growth 1/Gap 1 phase; G2 = Growth 2/Gap 2 phase; M = mitosis; MDM2 = murine double minute 2; NOXA = phorbol-12-myristate-13-acetate-induced protein 1, p21 = cyclin-dependent kinase inhibitor 1A (CDKN1A, CIP1); PUMA = p53 upregulated modulator of apoptosis; S = synthesis phase.

A. Autoregulatory feedback loop between p53 and its negative regulator MDM2.

B. MDM2 antagonist blocks p53-MDM2 binding; therefore, releasing p53 from negative control and activating the p53 pathway.

C. Stabilization and activation of p53 resulting in cell cycle arrest and apoptosis.

2.2 SCIENTIFIC RATIONALE FOR DEVELOPMENT OF RO5503781 TREATMENT IN ADULT AND PEDIATRIC INDICATIONS

RO5045337 (also known as RG7112) was the first nutlin to be used in clinical studies. Clinical evaluation of RO5045337 showed evidence of acceptable safety as well as clinical and biomarker activity in patients with solid tumors (Kurzrock et al. 2012; Ray-Coquard et al. 2012) and those with leukemia as a single agent and in combination with cytarabine (Andreeff et al. 2012; Yee et al. 2013). RO5045337 was not taken further in development due to potency and multi-gram dosing challenges; therefore, Roche is now focusing its clinical development program of MDM2 antagonists on the development of RO5503781 for patients with solid tumors and patients with leukemia.

RO5503781 is representative of a new generation of the nutlin family of MDM2 antagonists. In comparison with RO5045337, RO5503781 binds selectively to the p53 site on the surface of the MDM2 molecule in vitro with enhanced binding specificity and increased potency (Section 4), and has significantly reduced pill count requirements.

An important characteristic of several types of pediatric tumors is that loss-of-function mutations of the p53 tumor suppressor gene are uncommon, unlike many adult tumors (Van Maerken et al. 2014). Pediatric cancers with a pronounced incidence, including ALL, neuroblastoma, rhabdomyosarcoma, and Ewing's sarcoma, have a low frequency of *TP53* mutations (range: 1%–11%), while the most common adult cancers have a much higher frequency of *TP53* mutations (range: 23%–47%) (Van Maerken et al. 2014). Nonclinical studies have shown that nutlins are active against cell lines derived from a number of pediatric cancers, including ALL (Wada et al. 1993; Zhou et al. 2000; Bueso-Ramos et al. 1993; Marks et al. 1997; Gu et al. 2008; Zhou et al. 1995).

Nonclinical data predict that RO5503781 may be most effective in patients with tumors that have functional p53. Given the central importance of p53 activation in preventing carcinogenesis and the frequency of functional p53 in pediatric cancers, RO5503781 is believed to be a promising agent that may offer a new therapeutic option for pediatric cancers (Lane et al. 1999; Chene et al. 2003; Böttger et al. 1997; Vassilev et al. 2007).

3. REGULATORY HISTORY

In the United States, the Investigational New Drug application for RO5503781 was approved on 01 March 2013. An orphan drug application for RO5503781 in the treatment of AML was approved by the U.S. Food and Drug Administration (FDA) on 19 May 2014. Pediatric development plans for RO5503781 in the United States are being considered.

An application for orphan status for RO5503781 in the treatment of AML was approved by the European Commission on 22 August 2014. A positive opinion was issued on 12 September 2014 by the European Medicines Agency's (EMA) Paediatric Committee (PDCO) regarding the proposed Paediatric Investigation Plan (PIP).

Globally, studies of RO5503781 are being conducted across solid and hematologic tumors (Section 5).

RO5503781 is not approved for marketing in the United States or any other jurisdiction.

4. NONCLINICAL DATA SUPPORTING CLINICAL STUDIES

4.1 OVERVIEW

The three nutlin molecules developed by Roche, nutlin-3a, RO5045337, and RO5503781 (Table 1) all have the same mechanism of action and exhibit a similar on target toxicity profile as expected for selective MDM2 antagonists. Each successive molecule to be developed has demonstrated increasing potency, selectivity, and optimization of pharmacological properties as appropriate for clinical development. Thus, nonclinical data for all three molecules are presented in support of clinical development in adults and children; however, Roche is developing only RO5503781 for the treatment of adult and pediatric patients.

Nonclinical pharmacology studies show that RO5503781 binds MDM2, activates signaling downstream of p53, and induces p53-dependent apoptosis, providing proof-of-concept for the potential therapeutic application of MDM2 antagonists in several cancer indications, including those prevalent in pediatric populations harboring wild-type p53. Available animal and human safety data are considered sufficient to support the pediatric development of RO5503781 in advanced oncological disease in children $\geq$2 years of age. A juvenile toxicity study is planned to support pediatric trials in children from birth to $<$2 years of age (Section 7).

Table 1 List of Nutlin Molecules Developed by Roche

RO Number	Nomenclature Referenced in Public Domain	Chemical Class	Overview
—	nutlin-3a	Imidazoline	Research tool not suitable for the clinic. Available for nonclinical research.
RO5045337	RG7112	Imidazoline	First nutlin developed for clinical research. Discontinued for future development due to limited development potential.
RO5503781	RG7388	Pyrrolidine	Second nutlin developed for clinical research. New chemical class, optimized properties, and improved potency in comparison with RO5045337.

4.2 NONCLINICAL PHARMACOLOGY

4.2.1 RO5503781 Activates p53 In Vitro

RO5503781 is a potent and selective inhibitor of MDM2-p53 binding. In cell-free assays, RO5503781 binds to MDM2 protein with high affinity (dissociation constant [K_D] = 9.79 nM) and inhibits MDM2-p53 binding with IC_{50} of 6.0 nM (Ding et al. 2013).

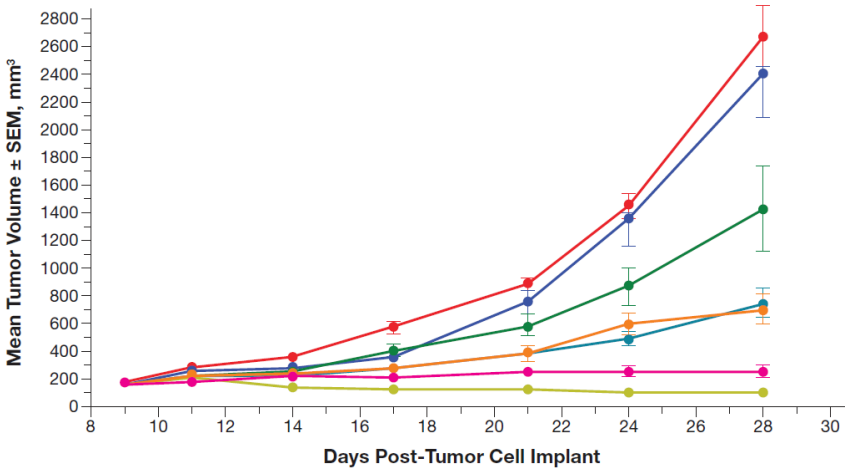
RO5503781 stabilizes and causes accumulation of activated p53 in vitro, causing inhibition of cell growth. RO5503781 induced dose-dependent p53 stabilization, cell cycle arrest, and apoptosis in cancer cells expressing wild-type p53 (Ding et al. 2013; Higgins et al. 2013; Higgins et al. 2014). Treatment of the MDM2-amplified osteosarcoma cell line SJSA-1 with RO5503781 showed a dose-dependent accumulation of p53 protein and its transcriptional targets, p21 and MDM2 (Higgins et al. 2013; Higgins et al. 2014). Further, treatment of SJSA-1 cells with RO5503781 for 24 hours led to a dose-dependent cell cycle block in G1 and G2/M phases, and a depletion of the S phase compartment (Higgins et al. 2013). Microscopic observation of arrested cells revealed flat morphology and a decrease of mitotic cells, indicating that the G2/M fraction consists of cells in G2 phase. Exposure of SJSA-1 cells to RO5503781 for 48 hours also led to induction of apoptosis in a dose-dependent manner (Higgins et al. 2013).

Nonclinical studies have indicated that tumors expressing wild-type p53 may respond to MDM2 antagonists; tumors with wild-type p53 and MDM2 amplification are likely to be the most sensitive. Cell lines with p53 mutation are uniformly less sensitive to MDM2 antagonists than those will wild-type p53 (Vassilev et al. 2007). RO5503781 inhibited proliferation to a greater extent in various solid tumor cells lines expressing wild-type p53 when compared to cancer cell lines with p53 mutation (Ding et al. 2013), demonstrating a 344-fold greater selectivity for cells with wild-type p53 versus mutant p53.

4.2.2 RO5503781 Demonstrates Anti-Tumor Activity In Vivo

In vivo, RO5503781 has shown anti-tumor activity at nontoxic doses against the SJSA-1 osteosarcoma xenograft model (Ding et al. 2013; Higgins et al. 2014). Daily and weekly oral administration of RO5503781 inhibited SJSA-1 human osteosarcoma xenografts in a dose-dependent manner, with $>$100% tumor growth inhibition (TGI) elicited after daily oral doses of 30 mg/kg RO5503781 (Figure 2) (Higgins et al. 2014).

Figure 2 RO5503781 Dose-Dependent Tumor Response in Osteosarcoma Xenograft Model



RG7388 Regimen	TGI[a], %	PR[b]	ILS, %
Vehicle	–	0	–
1.11 mg/kg qd	11	0	0
3.33 mg/kg qd	50	0	25
10 mg/kg qd	77	1	25
30 mg/kg qd	> 100	9	125
50 mg/kg qw	79	1	32
50 mg/kg 2x/ wk	96	3	57

ILS = increased life span; qd = once daily; qw = once weekly; PR = partial regression; TGI = tumor growth inhibition; wk = week.

Note: RG7388 = RO5503781.

[a] $P < 0.05$.

[b] N = 10 per group.

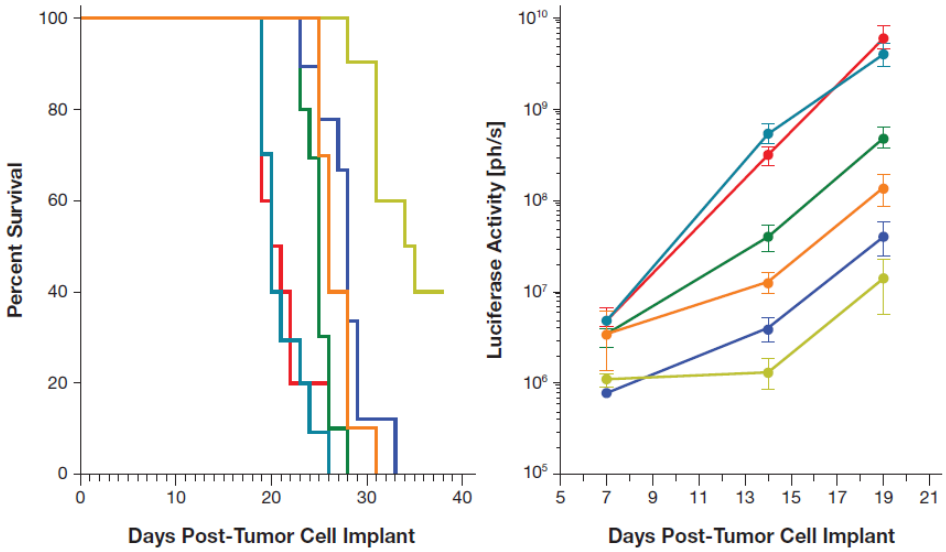
Sources: Higgins et al. 2013; Higgins et al. 2014.

4.2.3 MDM2 Antagonism is Synergistic with Cytotoxic and Androgen Ablation Therapy in AML and Prostate Cancer Models

MDM2 antagonists may synergize with chemotherapeutics that activate p53 signaling by means of the genotoxic stress imposed on the cells. Exposure of cultured leukemic cells (MOLM-13-luciferase.c4) to RO5503781 leads to dose-dependent accumulation of p53 protein and activation of its transcriptional targets and the p53 pathway. As a result, leukemic cells undergo a cell cycle block in G1 and G2 phases followed by apoptosis (Higgins et al. 2013). This allows for an increase in lifespan and decrease in luciferase activity in mice orthotopically implanted with the AML MOLM-13-luciferase.c4 model when treated with two optimal schedules of RO5503781 as monotherapy. The combination of these two optimal schedules of RO5503781 with cytarabine rendered

increased survival and decreased luciferase activity versus correlative monotherapy arms in this same model (Figure 3).

Figure 3 Combined Activity of RO5503781 and Cytarabine in the MOLM-13-luciferase.c4 AML Xenograft Model



Treatment Regimen[a]	ILS[b], %	*P* Value vs Vehicle Day 14	*P* Value vs Vehicle Day 19
Vehicle qw po/ iv	–	–	–
RG7388 80 mg/kg qd × 5 po	37	0.0013	0.0033
RG7388 100 mg/kg qw po	22	0.0024	0.0036
ARA-C, 200 mg/kg 2 ×/ wk iv	0	0.1658	0.0036
RG7388 80 mg/kg + ARA-C 200 mg/kg	68	0.0007	0.0019
RG7388 100 mg/kg + ARA-C 200 mg/kg	27	0.0010	0.0022

Ara-C = cytarabine; qd = once daily; qw = once weekly; d = day; po = oral; iv = intravenous; ILS = increased life span.

Note: MOLM-13-luciferase.c4 = acute myeloid leukemia cell line.

[a] Drug treatment started on Day 3.

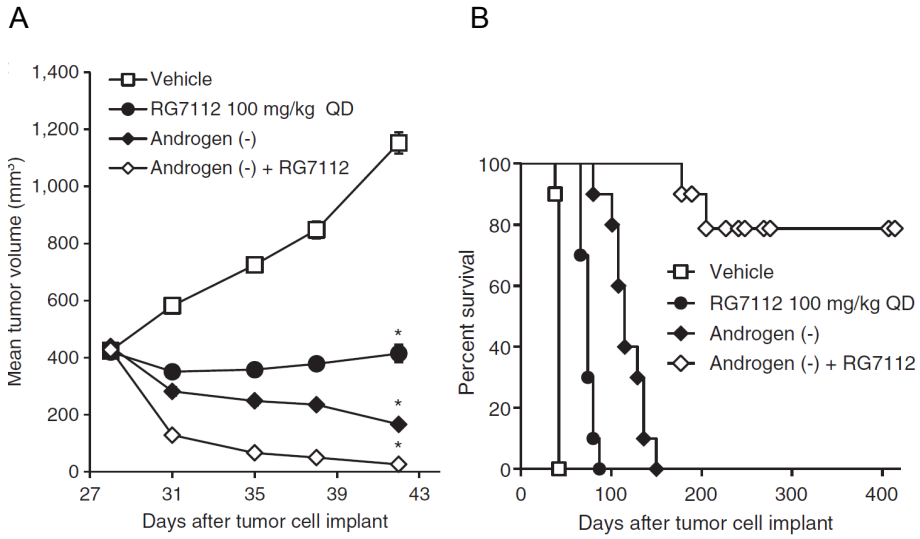
[b] Combinations groups survival was statistically longer than that in correlative monotherapy groups ($P < 0.05$).

Source: Higgins et al. 2014.

MDM2 antagonists have been shown to synergize with androgen ablation mice implanted with the androgen-dependent prostate cancer cell line LNCaP (Tover et al. 2011). Daily administration of 100 mg/kg RO5045337 or androgen withdrawal effectively inhibited tumor growth and induced partial tumor regression (Tover et al. 2013). Combined administration of 100 mg/kg RO5045337 and androgen withdrawal caused a marked tumor regression with virtually no palpable tumors evident at the end of the

two-week treatment period (Figure 4 A). When followed out after treatment ended, the lifespan was substantially increased in both monotherapy arms (76% for RO5045337; 174% for androgen withdrawal) (Figure 4 B). For the combined treatment with androgen withdrawal and RO5045337, however, markedly increased lifespan of greater than 800% was observed, indicating that the majority of mice died from natural causes rather than a return of tumor burden.

Figure 4 Combined Activity of RO5045337 and Androgen Ablation in an Androgen-Dependent Prostate Cancer Cell Line



QD = once daily.
Note: RG7112 = RO5045337.
Source: Tovar et al. 2013.

4.2.4 Efficacy in Tumor Indications Prevalent in Pediatric Populations

4.2.4.1 Pediatric Preclinical Testing Program

RO5045337 and its inactive enantiomer were evaluated by the Pediatric Preclinical Testing Program (PPTP) against an in vitro panel of 23 cell lines (Carol et al. 2013). Response outcomes were related to MDM2 and p53 expression datasets. Tumor growth inhibition meeting criteria for intermediate activity (event-free survival [EFS] tumor/control [T/C] > 2) was demonstrated in 10 of 26 (38%) solid tumor xenografts (Carol et al. 2013). Objective responses included medulloblastoma, alveolar rhabdomyosarcoma, Wilms, rhabdoid, and Ewing sarcoma xenografts. An ALL panel was also screened; there was one partial response, five complete responses, and one maintained complete response after single agent treatment with RO5045337. The authors concluded that this high level of activity supports prioritization of this compound for further evaluation (Carol et al. 2013).

A review of the nonclinical data supporting the use of MDM2 antagonists in pediatric tumor types is supportive of the potential therapeutic use of inhibition of p53-MDM2 interaction in neuroblastoma, rhabdomyosarcoma, and Ewing's sarcoma (Van Maerken et al. 2014). Additional select information is included below.

4.2.4.2 Neuroblastoma

Mutations in the *TP53* gene are very rare (~1%) in neuroblastoma tumors at diagnosis and are also rather uncommon (~15%) at relapse. At the cellular level, nutlin-3a-induced p53 activity does not only elicit proliferation arrest at the G1/S boundary of the cell cycle and massive apoptosis, but also premature senescence and neuronal differentiation in some neuroblastoma cell lines with wild-type *TP53* (Van Maerken et al. 2006). Oral administration of nutlin-3a to mice carrying chemo-resistant subcutaneous neuroblastoma xenografts with wild-type *TP53* was found to suppress both the growth of the primary neuroblastoma tumor and the extent of metastatic disease (Van Maerken et al. 2009). Evaluation of RO5503781 with pediatric collaborators has confirmed and extended these findings in neuroblastoma, with evidence of activity in vitro and in vivo xenograft models (Lakoma et al. 2014), as well as synergy with standard chemotherapeutics used in neuroblastoma (personal communication) and with targeted therapeutics (Van Goethem et al. 2014). In addition, gene set enrichment analysis and pathway analysis identified a set of p53-repressed genes that were reciprocally over-expressed in neuroblastoma patients with poor outcome (Barbieri et al. 2013). Multifactorial regression analysis identified a subset of four genes (*CHAF1A*, *RRM2*, *MCM3*, and *MCM6*) whose expression together strongly predicted overall and event-free survival ($P < 0.0001$).

4.2.4.3 Rhabdomyosarcoma

Mutations in *TP53* are present in 1.3%–5% of rhabdomyosarcoma tumors at diagnosis (Ognjanovic et al. 2012; Taylor et al. 2000) and in approximately 30% of rhabdomyosarcomas that have been previously exposed to chemotherapy. Nonclinical data show that this pediatric soft tissue sarcoma is highly sensitive to nutlin-3a. Treatment with nutlin-3a resulted in restoration of p53 function, growth inhibition, cell-cycle arrest, and apoptosis in rhabdomyosarcoma cell lines with wild-type *TP53* (Miyachi et al. 2009).

4.2.4.4 Ewing's Sarcoma Family Tumors

Approximately 89–90% of all Ewing's sarcomas retain wild-type *TP53*. Treatment of Ewing's sarcoma cell lines with nutlin-3a has been shown to activate the p53 pathway, to inhibit cell growth, and to induce apoptosis in a dose-dependent manner when wild-type *TP53* is present (Pishas et al. 2011; Sonnemann et al. 2011).

4.3 NONCLINICAL DRUG METABOLISM AND PHARMACOKINETICS

Single-dose pharmacokinetic (PK) studies demonstrated that high exposures were achieved in all nonclinical species after oral administration and high oral bioavailability was observed in rodents. Multiple-dose PK studies demonstrated that exposure increased with increasing dose level and no gender difference was apparent. In rats, there was generally no accumulation or loss of exposure after multiple days of dosing. However, after repeat dosing of RO5503781 in monkeys, decreases in exposure were apparent at all dose levels, which correlates to monkey-specific CYP3A induction in intestine and liver.

Tissue distribution studies in rats showed that RO5503781 was widely distributed and the highest concentration was observed approximately 4 hours postdose in intestine, liver, adrenal gland, colon, cecum, kidney cortex, and gastric mucosa. Based on in vitro results, both rats and monkeys produced the same metabolites as humans. In rats in vivo, [^{14}C]-RO5503781 and its related metabolites were mainly excreted hepatically through bile with minimal renal elimination.

RO5503781 is metabolized by multiple metabolic enzymes, mainly by CYP2C8, CYP3A4/5, and UDP-glucuronosyltransferase; strong inhibitors and/or inducers of these enzymes may affect exposure of RO5503781.

4.4 NONCLINICAL SAFETY

Pivotal repeat-dose cycling studies in rats and monkeys confirmed that RO5503781 produced target organ toxicities expected for a compound that disrupts the cell cycle. Safety findings identified at exposures that approximate the anticipated therapeutic range are considered clinically manageable (e.g., diarrhea), monitorable (e.g., suppression of blood cell components), and/or reversible (e.g., body and organ weight changes and diarrhea). No biologically relevant adverse effects were observed in the central nervous system (CNS) or pulmonary system. Cardiovascular effects were limited to a reversible increase in heart rate in male monkeys at doses above the maximum-tolerated dose (MTD); however, there was no effect on the corrected QT interval (QTc) in the pivotal monkey study at doses that exceeded the MTD. RO5503781 poses little to no risk of phototoxicity in cancer patients. In vitro and in vivo genotoxicity assays were negative.

5. CLINICAL TRIAL EXPERIENCE IN ADULT PATIENTS

5.1 OVERVIEW

Phase I/Ib assessment of RO5503781 is ongoing in three studies, as follows:

- Study NP27872: Phase I entry-into-human study in patients with solid tumors (completed; final results not yet available) (Siu et al. 2014)
- Study NP28679: Phase I/Ib dose-finding study in combination with cytarabine in patients with AML (Yee et al. 2014)
- Study NP28902: Phase I drug-drug interaction, relative bioavailability, and food effect of a new formulation study in patients with solid tumors

Promising hematological malignancy responses have been observed in a population of relapsed/refractory AML patients treated with RO5503781 in combination with cytarabine in Study NP28679. Extension phases are ongoing due to the rapid and durable responses observed in the context of an acceptable safety profile, consisting mainly of gastrointestinal and hematopoietic toxicities. Findings from these clinical trials will provide data that will inform the study of pediatric populations, which are initially planned for pediatric acute leukemias (ALL and AML) and solid tumors.

5.2 STUDIES IN PATIENTS WITH SOLID TUMORS

5.2.1 Study NP27872

Study NP27872 is an open-label trial of RO5503781 monotherapy in patients with advanced malignancies. Three RO5503781 dosing schedules (once weekly $\times$ 3, daily $\times$ 5, or daily $\times$ 3) given on a planned 28-day schedule were assessed to determine safety and tolerability, and the food-effect and biomarker activity of RO5503781 were also investigated. The study is now completed. Data from a preliminary analysis for 95 of 99 enrolled patients were presented at the 2014 American Society of Clinical Research Annual Meeting (Siu et al. 2014).

Dose escalations were performed independently for weekly versus daily dosing schedules. The MTD per schedule was: 1600 mg BID once weekly $\times$ 3, 500 mg daily $\times$ 5, and 500 mg BID $\times$ 3. The dose-limiting toxicities (DLTs) differed between weekly and daily dosing schedules, with nausea and vomiting DLTs associated with the weekly administration, while more frequent myelosuppression DLTs defined the MTD in the daily administration schedules. Myelosuppression was also associated with PK exposure.

Pharmacodynamic biomarker macrophage inhibitory cytokine-1 (MIC-1) activation was observed at the lowest dose evaluated. Preliminary biomarker results suggest that p53 activation was greater with daily $\times$ 5 dosing than with other schedules at the respective MTDs. The best response was stable disease in sarcoma patients, notably 17, 22, and $>$ 23 months.

In summary, this study demonstrated RO5503781 proof-of-mechanism, p53 activation, which may be dose- and schedule-dependent; therefore, further evaluation is warranted.

5.2.2 Study NP28902

Study NP28902 is a multicenter, open-label, crossover design, clinical pharmacology study to investigate RO5503781 drug-drug interaction with posaconazole (Part 1) and relative bioavailability of new formulations of RO5503781 (Part 2), and food-effect on the pharmacokinetics of a single dose with the optimized solid dispersion formulation (Part 3) in patients with solid tumors. Parts 1 and 2 are now completed. Preliminary analysis suggests that there is no significant potential for RO5503781 drug-drug interaction with strong CYP3A4 inhibitors, and a newer version of the solid dispersion formulation demonstrated better relative bioavailability in comparison with the current Phase I formulation. Part 3 is now open for enrollment.

5.3 STUDIES IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

5.3.1 Study NP28679

An extension phase of Study NP28679, the open-label, Phase I/Ib dose-escalation study of RO5503781 in combination with cytarabine in patients with AML is currently ongoing following the promising efficacy observed in the early phase of the trial.

Dose escalations of RO5503781 alone (Part 1, $N = 20$) and in combination with cytarabine (Part 2, $N = 23$) are now completed. RO5503781 was generally tolerated in this population of mostly pretreated, older patients (median age 68.5 years in Part 1, 64 years in Part 2). One DLT of prolonged myelosuppression was reported; however, the recommended Phase 2 dose (RP2D) was determined by tolerance-limiting diarrhea not formally established as a DLT.

An extension of Part 1 (single agent RO5503781 as first-line therapy of patients not suitable for standard induction therapy as defined by age [> 70 years] and/or comorbidities [≤ 70 years]) was discontinued after the first 9 patients were enrolled due to the risk of infection and early death associated with prolonged myelosuppression. Three patients (all age ≥ 75 years) died in the first 30 days; causes of death included disease progression and infection-related complications.

An extension of Part 2 (combination of RO5503781 with or without cytarabine in relapsed or refractory AML) was also performed. Because patients with relapsed or refractory disease present with neutropenia and thrombocytopenia due to underlying leukemic marrow infiltration, they are already at risk for infectious and bleeding complications before trial initiation, including neutropenic infections (i.e., bacterial, fungal), sepsis, and hemorrhage. At trial entry, patients who are already at risk of bleeding and colonized with potential pathogens are further challenged with a period of aplasia caused by RO5503781 treatment. These patients are at significant risk for

life-threatening complications during trial participation. To help manage these risks, Roche further defined with investigators the importance of excluding patients with active infections.

Overall, the current safety profile of RO5503781 in Study NP28679 is consistent with findings from other studies of relapsed/refractory AML patients treated with cytarabine combination regimens. The most common adverse events were gastrointestinal- (diarrhea in >85% of patients) and infection-related (>70% of patients). The assignment of causality for the observed events is complicated by common comorbidities and progression of disease in AML, which often overlap with potential drug effects.

Rapid hematological responses were observed in elderly AML patients treated with RO5503781 monotherapy and in those with relapsed/refractory disease treated with cytarabine combination therapy (Table 2). Across all study groups there were 13 complete remissions (CRs), 5 complete remissions with incomplete recovery of peripheral counts/morphological leukemia-free state (<5% marrow blasts without recovery of counts; CRi/MLFS), 6 partial responses (PRs), and 9 hematological improvements (multiple cycles, decreased peripheral/marrow blast; HIs) among 65 evaluable patients. CRs were observed in diverse patients, including those with varied risk groups, prior antecedent hematological disorders (AHD), therapy-related AML (t-AML), and relapsed/refractory and de novo AML. Additionally, 1 patient with a mutation in the protein coding domain of *TP53* achieved CR. Complete responses were durable as these patients had a subsequent bone marrow assessment that confirmed continued bone marrow response approximately 28 days following the initial CR assessment. All patients who achieved a CR during dose escalation were relapse-free for >60 days. One patient treated with RO5503781 monotherapy and 2 with combination therapy have remained relapse-free for >400 days and >200 days from study start, respectively.

A potential predictive gene expression signature derived from assay of pretreatment specimens with RO5045337 has shown association with MDM2 antagonist therapeutic response in an earlier trial of previous generation MDM2 antagonist therapy. Current statistical analysis indicates significance with RO5503781 therapeutic response (area under the receiver operator characteristic [AUROC]=73%, P=0.021) in NP28679 Part 1 and 2 dose-escalation patients. This biomarker strategy with the potential for response discrimination will undergo continued validation assessments in extension patients as well as upcoming clinical trials (Zhong et al., unpublished data).

An optimized formulation of RO5503781 expected to have higher exposure and allow more patients to achieve an efficacious dose has been developed. This optimized solid dispersion formulation is being administered at dose levels up to the RP2D in combination with cytarabine in Part 4 of Study NP28679 to characterize the safety profile of RO5503781.

Preliminary results demonstrate activity of RO5503781 monotherapy in AML patients with or without prior treatment history for hematologic diseases and promising activity of RO5503781 in combination with cytarabine in patients with relapsed/refractory AML who were previously treated. This study has demonstrated proof-of-concept, promising efficacy, and acceptable risk/benefit for further evaluation of RO5503781 in combination with cytarabine in AML patients with relapsed/refractory disease. Roche is currently in discussion with Heath Authorities globally to determine how best to advance the clinical development program.

Table 2 Study NP28679–Patient Characteristics by Response to Treatment

Monotherapy Dose Escalation (Part 1, N=20; 16 evaluable responses)				
Best response	2 CR; 3 CRi/MLFS	3 PR	4 HI	4 PD
TP53 status	WT	WT	1 MT, 3 WT	1 V
ELN [a]	4 I, 1 A	3 I		
AHD (responders)	3 (MDS, MF, CMML)	2 (MDS, CMML)		
Monotherapy Extension (Part 1 Extension, N=9; 6 evaluable responses)				
Best response	1 CRi/MLFS	0 PR	2 HI	3 PD
TP53 status	WT		WT, MT	2 WT, 1 U
ELN [a]	I			
AHD (responders)	ET			
Combination with Cytarabine Dose Escalation (Part 2, N=23; 20 evaluable responses)				
Best response	6 CR	2 PR	2 HI	10 PD
TP53 status	1 MT	WT	WT	1 MT, 1 V
ELN [a]	1 F, 3 I, 2 A	1 I, 1 A		
AHD (responders)	1 t-AML			
RO5503781 ± Cytarabine (Part 2 Extension, N=34; 23 evaluable responses)				
Best response	4 CR; 1 CRi/MLFS	1 PR	1 HI	16 PD
TP53 status	4 WT, 1 U	WT	WT	1 MT, 5 WT, 10 U
ELN [a] (responders)	1 F, 3 I, 1 A	A		

CR=complete remission; CRi=complete remission with incomplete recovery; HI=hematologic improvement; MLFS=morphologic leukemia-free state; PD=progressive disease; PR=partial response; t-AML=therapy-related AML; WT=wild-type; V=splice variant; U=unknown/pending; AHD, includes myelodysplastic syndrome (MDS), essential thrombocythemia (ET), chronic myelomonocytic leukemia (CMML), myelofibrosis (MF).

[a] ELN: Risk by European Leukemia Net: favorable (F), Intermediate I and II (I), or adverse (A).

5.4 CLINICAL PHARMACOLOGY

Preliminary clinical PK data from patients with solid tumors indicates that RO5503781 dose-PK exposure (AUC at steady–state) was proportional up to 2400 mg/day, and the half-life was approximately 1 day. The reported dose-exposure relationship was consistent with preliminary results of RO5503781 in patients with AML. The pharmacokinetics did not vary with older age, body weight, Asian ethnicity, concomitant azoles, or cytarabine treatment. Bone marrow levels were approximately 70% of plasma drug levels at steady-state. There was no clinically significant effect of high-fat food observed with PK exposure; low-fat food does not appear to impact PK. The drug is mainly metabolized to inactive metabolites and not excreted in the urine. Strong inhibition of CYP3A4 has no clinical significance on PK parameters. Lastly, there was no evidence of plasma concentration-related change in Fridericia's corrected QT interval (QTcF).

6. CURRENT DRUG DEVELOPMENT PLAN FOR OTHER INDICATIONS IN ADULTS

Research activities and collaborations are focused on expanding the development of RO5503781 in hematological cancers and solid tumors for which a strong scientific rationale is evident. RO5503781 may have utility in a number of tumor types in combination with standard-of-care treatments on the basis of its mechanism of action. The development of RO5503781 in these adult indications will further inform the proposed development plan in pediatric patients.

There are planned or ongoing investigations for RO5503781 in disease areas such as myeloproliferative neoplasms, metastatic castration-resistant prostate cancer, and multiple myeloma in collaboration with external groups.

7. CURRENT CLINICAL DEVELOPMENT PLAN IN PEDIATRICS

7.1 OVERVIEW

While there are improved treatment agents for children with primary cancers, relapsed patients have fewer therapeutic options and poorer outcomes. There is a high unmet medical need for pediatric patients with ALL and AML that have relapsed/refractory disease, and clinical studies in adults support the therapeutic potential of RO5503781 in children with these diseases. A strong scientific rationale also exists for the therapeutic potential of RO5503781 in prevalent pediatric solid tumors because the majority of pediatric tumors are wild-type for *TP53* (Pinto et al. 2011). An international collaborative research consortium of investigators has partnered with Roche because of the relevance of RO5503781 for the treatment of pediatric cancers. This consortium has collaborated to provide supportive nonclinical evidence for RO5503781 pediatric development. These collaborative efforts have enabled extension of Roche's pediatric development plan to indications beyond those planned for adults, such as neuroblastoma, rhabdomyosarcoma, and Ewing's sarcoma. The main challenges identified for pediatric development include the selection of patients most likely to benefit from RO5503781 treatment (i.e., those harboring functional p53 given anecdotal context that some mutant alleles remain therapeutically responsive), the variability in exposure observed in adult patients treated with RO5503781, and the development of a formulation suitable for children.

7.2 EPIDEMIOLOGY

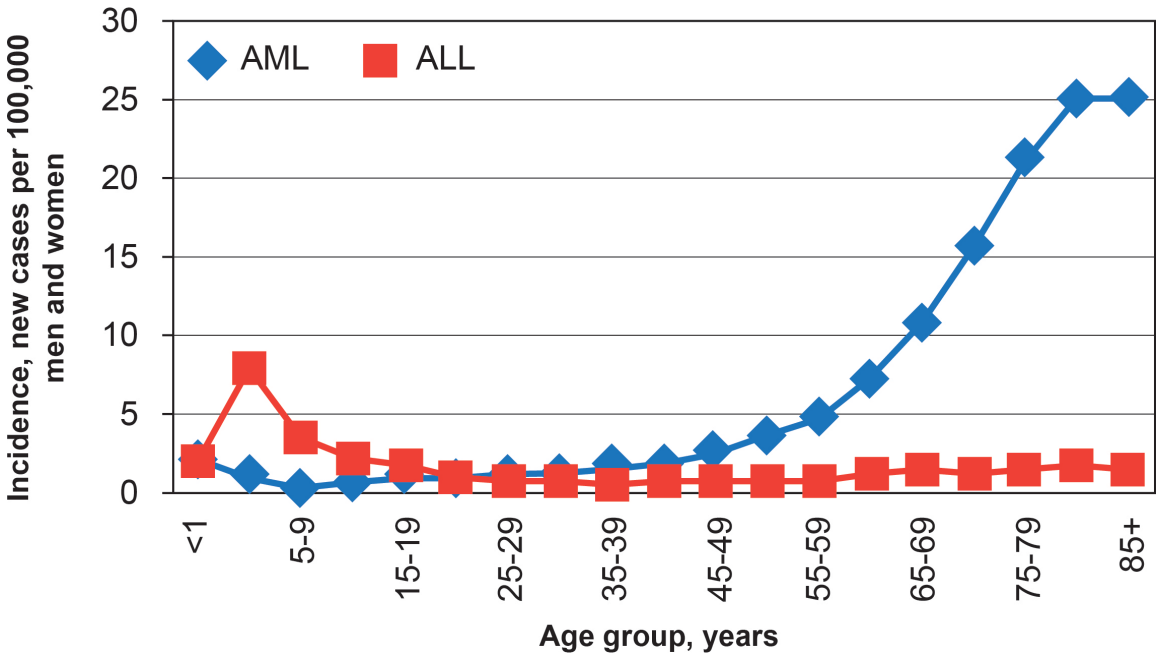
7.2.1 Pediatric Acute Lymphocytic Leukemia and Acute Myeloid Leukemia

While all pediatric cancers are rare, ALL is the most common pediatric cancer, with an overall incidence rate of 35.1 per 1,000,000 (ages 0–19) in the United States (SEER 2012) (Table 3), with a peak incidence in children 1 to 4 years of age (Figure 5). Based on Surveillance Epidemiology and End Results (SEER) data from 2006–2010, the median age of diagnosis for ALL was 14 years of age in the United States and approximately 59.5% of patients with ALL were diagnosed prior to age 20 years. Approximately 18.1% of ALL patients under 20 years of age in the United States died. The annual age-adjusted mortality in the United States based on patients with ALL (all ages) who died from 2006 to 2010 is 0.5 deaths per 100,000 (SEER 2013).

AML is more common in adults than in the pediatric population, although there is a peak in the occurrence of cases in early childhood (≤ 1 year). The age-adjusted incidence of AML in the United States was approximately 8.3 cases per 1,000,000 children (ages 0–19) (SEER 2012). On the basis of SEER data from 2006 to 2010, the median age at diagnosis for AML was 67 years of age in the United States. An estimated 700 new cases are expected to occur in pediatric patients annually in the United States.

Although treatment outcomes have improved, a significant number of pediatric ALL and AML patients relapse (17.5% and 12%–38%, respectively), so there remains a high unmet medical need.

Figure 5 Annual Age-Adjusted Incidence of AML and ALL in the United States, 2006–2010



Source: Surveillance Epidemiology and End Results (SEER)*Stat Database: Incidence - SEER 18 Regs Research Data + Hurricane Katrina Impacted Louisiana Cases, Nov 2012 Sub (2000–2010) <Katrina/Rita Population Adjustment> - Linked To County Attributes - Total U.S., 1969–2011 Counties, National Cancer Institute, DCCPS, Surveillance Research Program, Surveillance Systems Branch, released April 2013, based on the November 2012 submission.

Table 3 Key Characteristics of Pediatric Patients with AML and ALL, and Estimated Number of New Pediatric Patients per Year in the United States and Europe

				United States			Europe		
Tumor Type	Age at Peak Incidence, Pediatrics (years)	Relapse (%)	Median Time from Diagnosis to Relapse	Newly Diagnosed Rate (cases per million) [a]	n per year (rate × n population) [b]	Relapse [c] (n)	Newly Diagnosed Rate (cases per million) [d]	n per year (rate × n population) [b]	Relapse [c] (n)
ALL	1–4	20 [e]	2.3 years [e]	35.1	2949	590	29.5	4641	928
AML	≤ 1	12-38 [f]	9-12 months [f]	8.3	700	84-266	6.6	1045	125-397

ALL = acute lymphocytic leukemia; AML = acute myeloid leukemia.

Note: Roche consulted the literature to estimate the proportion of patients who relapsed.

[a] For the United States, the incidence was determined from the SEER cancer registries 2006–2010 (SEER 2012).

[b] To obtain the number of new patients per year (N), the Sponsor multiplied the incidence rates of these selected tumors by the total population of children in Europe or the United States.

[c] Estimates of the number (n) of relapsed patients were calculated by multiplying the total number of patients with the cancer by the proportionof patients that will have relapsed based on literature reports.

[d] For Europe, incidence was determined from data collected in 1988–1997 as part of the International Agency for Research on Cancer's Automatic Childhood Cancer Information System (ACCIS). A weighted average of age-specific rates reported by ACCIS was calculated to obtain a rate for 0–19 year olds.

[e] Ko et al. 2010; Pichler et al. 2013; Saarinen-Pihkala et al. 2006.

[f] Rubnitz et al. 2010; Sander et al. 2010; Abrahamsson et al. 2007; Aladjidi et al. 2003; Perel et al. 2002.

7.2.2 Pediatric Malignant Solid Tumors

On the basis of its mechanism of action, RO5503781 may have broad applicability in a number of pediatric cancers not harboring loss-of-function *TP53* mutations (Table 4).

The following tumor types all have peak incidences during childhood: neuroblastoma, osteosarcoma, Ewing's sarcoma, and rhabdomyosarcoma (Figure 6). Approximately 7% of pediatric cancers (ages 0–14) are neuroblastomas, approximately 4% are Ewing's sarcoma, and approximately 3% are rhabdomyosarcoma (ACS 2014). The key patient characteristics of these pediatric cancer indications, including age at peak incidence and time from diagnosis to relapse, are presented in Table 5. An estimated 644, 401, 225, and 410 new cases of neuroblastoma, osteosarcoma, Ewing's sarcoma, and rhabdomyosarcoma, respectively, are expected to occur in children annually in the United States.

Table 4 *TP53* Mutation Frequency in Pediatric Cancers

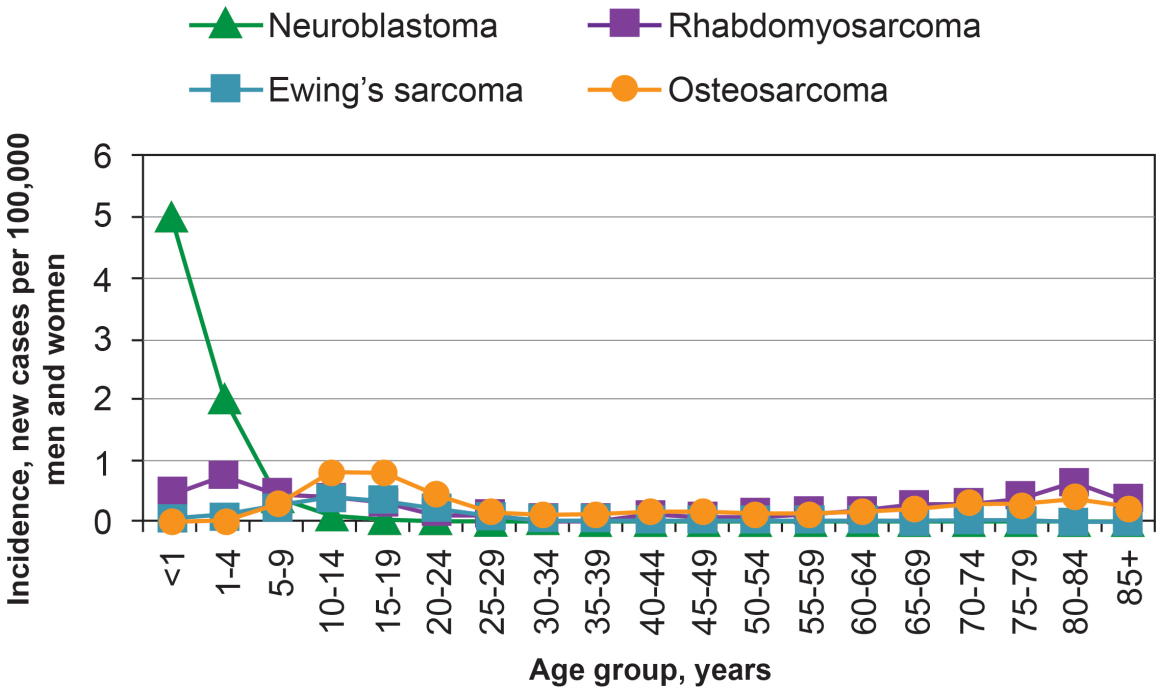
Tumor	*TP53* Mutation Frequency (%)
Neuroblastoma	1
Retinoblastoma	3
Osteosarcoma	19
Ewing's sarcoma	11
Rhabdomyosarcoma	1.3%–5% [a]
Medulloblastoma	6
Pediatric leukemia	2
Relapsed leukemia	12.7% [b]
Wilm's tumor	10
Pediatric glioma	1

[a] Ognjanovic et al. 2012; Taylor et al. 2000.

[b] Samples from adults and children analyzed. Zhu et al. 1999.

Source: Van Maerken et al. 2014.

Figure 6 Annual Age-Adjusted Incidence of Pediatric Solid Tumor Cancer Indications in the US (2006–2010)



Source: Surveillance Epidemiology and End Results (SEER)*Stat Database: Incidence - SEER 18 Regs Research Data + Hurricane Katrina Impacted Louisiana Cases, Nov 2012 Sub (2000–2010) <Katrina/Rita Population Adjustment> - Linked To County Attributes - Total U.S., 1969–2011 Counties, National Cancer Institute, DCCPS, Surveillance Research Program, Surveillance Systems Branch, released April 2013, based on the November 2012 submission.

Table 5 Key Characteristics of Pediatric Patients with Neuroblastoma, Rhabdomyosarcoma, Ewing's sarcoma, and Osteosarcoma, and Estimated Number of New Pediatric Patients per Year in the United States and Europe

				United States			Europe		
Tumor Type	Age at Peak Incidence (years)	Relapse (%)	Median Time from Diagnosis to Relapse	Newly Diagnosed Rate (cases per million) [a]	n per year (rate × n population) [b]	Relapse [c] (n)	Newly Diagnosed Rate (cases per million) [d]	n per year (rate × n population) [b]	Relapse [c] (n)
Neuroblastoma	≤ 1 year [e]	26-41 [e]	13 months [e]	7.7	644	167–264	10.9	1243	323–510
Rhabdomyosarcoma	0 – 4 years	28 [f]	6–12 months [g]	4.8	410	115	5.4	616	172
Ewing's sarcoma	10-14 years	30 [h]	13 months [i]	2.8	225	69	2.8	445	133
Osteosarcoma	10-14 years	21 [j]	16.5 months [k]	5.0	401	87	4.3	684	144

Table 5 Key Characteristics of Pediatric Patients with Neuroblastoma, Rhabdomyosarcoma, Ewing's sarcoma, and Osteosarcoma, and Estimated Number of New Pediatric Patients per Year in the United States and Europe (cont.)

Note: Roche consulted the literature to estimate the proportion of patients who relapsed.

[a] For the United States, the incidence was determined from the SEER cancer registries 2006–2010 (SEER 2012).

[b] To obtain the number of new patients per year (N), the Sponsor multiplied the incidence rates of these selected tumors by the total population of children in Europe or the United States.

[c] Estimates of the number (n) of relapsed patients were calculated by multiplying the total number of patients with the cancer by the proportion of patients that will have relapsed based on literature reports.

[d] For Europe, incidence was determined from data collected in 1988–1997 as part of the International Agency for Research on Cancer's Automatic Childhood Cancer Information System (ACCIS). A weighted average of age-specific rates reported by ACCIS was calculated to obtain a rate for 0–19 year olds.

[e] The highest incidence is among those of <1 year of age; however, the unmet need is among patients of 1–4 years of age; Haupt et al. 2010; London et al. 2011.

[f] Bisogno et al. 2012.

[g] Mattke et al. 2009.

[h] Potratz et al. 2012.

[i] McTiernan et al. 2006.

[j] Sampo et al. 2011.

[k] Stahl et al. 2011.

7.3 CURRENT STANDARD OF CARE IN PEDIATRIC LEUKEMIA AND/OR SOLID TUMORS

Chemotherapy, comprising initial induction therapy to promote CR followed by consolidation therapy, is curative only in subsets of pediatric leukemias (Pui and Evans 2006). The composition of the induction chemotherapy regimen used varies between indications; most frequently vincristine, corticosteroid, and L-asparaginase are used for standard-risk ALL patients, and four-drug induction regimens are standard for ALL at some institutions (Escherich et al. 2013). Most (>95%) patients will achieve CR within 4 weeks of therapy, and this treatment is followed by consolidation therapy comprising the Berlin-Frankfurt-Münster (BFM) backbone (Möricke et al. 2010). Children with AML are treated with cytarabine and an anthracycline as induction therapy and intensive post-remission therapy induction agents, non-cross–resistant drugs and high-dose cytarabine. Radiation therapy is also part of the therapeutic armamentarium for treating high-risk leukemias at risk for CNS dissemination.

The mainstays of treatment for solid tumor malignancy are chemotherapy, surgery, and radiation therapy. Different cancers are responsive to different agents and show varying degrees of susceptibility to radiation. Surgery alone is feasible and curative only in a minority of pediatric cancers (Pizzo et al. 2010) as micrometastatic disease is presumed to be present at the time of presentation or because the tumor is not completely resectable without unacceptable morbidity to adjacent tissues or organs. Hence, neoadjuvant or adjuvant chemoradiation therapy is mandated in a majority of advanced pediatric solid tumors. In most tumor types that present initially or secondarily with metastases, the prognoses are much worse and are more difficult to treat with focal modalities such as surgery and radiation. While the overall survival in children with cancer has improved tremendously over the past 40 years and is now close to 70% (figures in U.S., 75%), efforts must now focus on relapsed and refractory tumors. Relapse is a significant negative prognostic factor in the survival of pediatric patients with cancer. Relapse during initial treatment remains only rarely a survivable event (Grunewald et al. 2012). Furthermore, following completion of definitive therapy, relapse precludes survival in the vast majority of children with malignancies.

Five-year overall survival rates are less than 25% in diseases such as brain tumors or neuroblastoma that relapse after completion of initial therapy. As such, the focus of treatment in these children is on targeted therapies. Therapies such as RO5503781, which target critical molecular checkpoints involved in providing survival benefits to tumor cells, may prove particularly useful in children where conventional chemotherapies have rate-limiting or even unacceptable side effects on non-target organs. If proven effective and safe in the relapsed setting, targeted inhibitors such as RO5503781 could be moved to the therapeutic armamentarium applied to the first-line setting in an effort to de-escalate the dose intensity of conventional chemoradiotherapy approaches and limit their immediate and long-term side effects.

7.4 PEDIATRIC NONCLINICAL EVALUATIONS AND COLLABORATIONS

There has been long standing interest in the development of RO5503781 for pediatric cancers due to its suitable mechanism of action and given the high prevalence of cancers with functional p53 for the pediatric cancer setting. The PPTP, which was established to identify new agents with potential activity in pediatric cancers (Houghton et al. 2007), selected first-generation nutlin RO5045337 for systematic in vitro and in vivo testing on the basis of its potential efficacy for patients with functional p53 (Carol et al. 2013). Response outcomes were related to *MDM2* and *TP53* expression, and in vivo studies demonstrated tumor growth inhibition in solid tumor and ALL xenografts.

External collaborations have enabled the extension of Roche's pediatric development plan to indications beyond those planned for adults. Following an Investigator Meeting to discuss the findings of the PPTP, an international collaborative research consortium of investigators and Roche was established with a mandate to continue developing supportive nonclinical data for relevant pediatric indications, such as neuroblastoma and ALL (Section 4.2.4).

7.5 SELECTION OF PEDIATRIC INDICATION

The proposed pediatric indications are as follows:

RO5503781 is indicated for the treatment of children with first relapse of, or with frontline-refractory ALL; or

RO5503781 is indicated for the treatment of children with first relapse of, or with frontline-refractory AML; or

RO5503781 is indicated for the treatment of children with a solid tumor which is newly-diagnosed and metastatic, or refractory to first-line treatment.

7.6 PROPOSED STUDIES IN PEDIATRICS

7.6.1 Nonclinical Development Plan

Available animal and human safety data are considered sufficient to support the pediatric development of RO5503781 in advanced oncological disease in children $\geq$2 years of age. A juvenile toxicity study in rats of at least 7 days of age with focus on potential target organs (hematopoietic, lymphoreticular, gastrointestinal, and liver) is planned to support pediatric trials in children from birth to $<$2 years of age.

7.6.2 Clinical Development Plan

The initial clinical plans for development of RO5503781 in both adults and children are for relapsed/refractory disease. RO5503781 may have utility in combination with standard-of-care treatments in earlier lines of therapy, including initial therapy in both leukemia and solid tumors; however, based on safety concerns, particularly on-target myelotoxicity, expansion into earlier lines of treatment will be tested only after safety is

established in the relapsed/refractory setting. These data will inform future considerations in the pediatric population.

The clinical development plan includes the following:

- A pediatric study to evaluate the pharmacokinetics, toxicity, safety and any activity of RO5503781 in children with a solid tumor or acute leukemia
- For the purposes of deciding to advance to pivotal trial, a target treatment effect will be pre-specified for each disease, and will comprise both efficacy and safety criteria. With respect to efficacy, this effect for a particular disease will reflect a clinically meaningful improvement, as defined by expert consensus and health authorities. Ongoing communication and collaboration with academic collaborators and health authorities will be critical to the development of successful and meaningful target treatment effect criteria, to ensure that the pre-specified effect is optimally defined (and potentially redefined) as information accrues.

7.6.2.1 Dose-Finding Study

The first planned pediatric study is an open-label, non-controlled, multicenter trial to evaluate the pharmacokinetics, toxicity, safety, and any activity of RO5503781 in children from 2 years to less than 18 years of age with a solid tumor or acute leukemia for which no effective treatment is known, with expansion cohorts in defined subsets using RO5503781 combined with anti-cancer medicine(s). The dose-escalation phase to define the MTD will use a rolling six design (Skolnik et al. 2008), and the expansion cohorts will be opened following determination of the optimal dose only after analysis of gene expression and pharmacodynamics assessments from the dose escalation are completed.

The primary endpoint is safety, including monitoring intensified for any cardiovascular toxicities; the main secondary endpoints are as follows: PK parameters, response, pharmacodynamic assessment of biomarkers, *TP53* mutational status, and acceptability including palatability. At least 10 evaluable children will be enrolled in the dose-escalation cohorts, and at least 10 evaluable children per cohort will be enrolled in each expansion cohort.

7.6.2.2 Randomized Controlled Study

Roche believes that RO5503781 will ultimately provide a benefit to some children with cancer. Dependent on safety outcomes and early evidence of efficacy from the pediatric dose-escalation trial, a pediatric tumor type may also be identified for an additional therapeutic confirmatory study.

Such a study would be an open-label, randomized, controlled add-on trial to evaluate the safety and efficacy of RO5503781 in children from birth to less than 18 years of age with a first relapse of, or with frontline-refractory acute leukemia (either lymphoblastic or

myeloid) or solid tumor. A non-controlled run-in for safety of RO5503781 in combination would be incorporated unless results with combination are available.

The planned primary endpoint will be event-free survival; the main secondary endpoints PK assessments, pharmacodynamics assessment of biomarkers, *TP53* mutational status, and acceptability including palatability. Sample size will be determined on the basis of available data from the dose-finding study.

Preliminary combination data (including dose-finding) will be obtained in the Phase I study as an extension. The nature and number of the combination partner(s) will be based on preliminary signs of activity. This may include combination with standard-of-care or with targeted molecules for which there is a scientific rationale. Nonclinical data to support the appropriate combinations is currently being generated for pediatric tumor types, and information on safety and dosing from the adult programs will also be used to guide the appropriate choice of combinations for the extensions.

Identification of a tumor type susceptible to MDM2 inhibition with RO5503781 is an important feature of the proposed clinical trial. Investigation of the safe and effective combination of RO5503781 with chemotherapeutic agents in adults with cancer is ongoing and preliminary results are expected prior to initiation of a combination trial in pediatric patients. When information is available from the Phase I study, Roche will be able to determine the indication and appropriate combination partners for a confirmatory trial with RO5503781, and more precise details of the study can be provided.

7.7 CURRENT OR POTENTIAL CHALLENGES THAT HAVE BEEN IDENTIFIED REGARDING CLINICAL TRIALS IN CHILDREN

Roche has identified three potential challenges for the pediatric development of RO5503781 in patients with pediatric leukemias and solid tumors, as follows:

- Although many pediatric oncology patients are suitable for RO5503781 treatment due to the high prevalence of functional p53 in pediatric cancers, patients with non-functional p53 may not benefit from this treatment; however, anecdotal evidence shows that some mutant alleles remain therapeutically responsive. A patient selection strategy will determine which pediatric patients will derive the most benefit from RO5503781 treatment.
- Clinical studies of adult patients treated with RO5503781 have demonstrated variability in PK exposure. The impact of this variability on pediatric patients must be considered.
- The poor solubility of RO550381 and the sensitivity of its amorphous state to moisture have prevented the development of a liquid age-appropriate formulation of RO5503781.

7.7.1 Biomarker Development Plan and Results

Biomarker development is an integral aspect of the RO5503781 clinical development plan. The main challenge identified for biomarker development in the context of pediatrics and adults is the selection of patients most likely to benefit from RO5503781 treatment. To this end biomarker analyses in adult studies of RO5503781 have addressed two goals: 1) to confirm clinical activity via the intended/expected therapeutic mechanism of action (on-target activity) by determining change over baseline in biomarker measurements between patients following therapy administration compared with pre-dosing measurements; and 2) to discriminate likely responders from non-responders to RO5503781 treatment based upon measurement of analyte(s) in pre-treatment blood/plasma, or bone marrow-derived specimens.

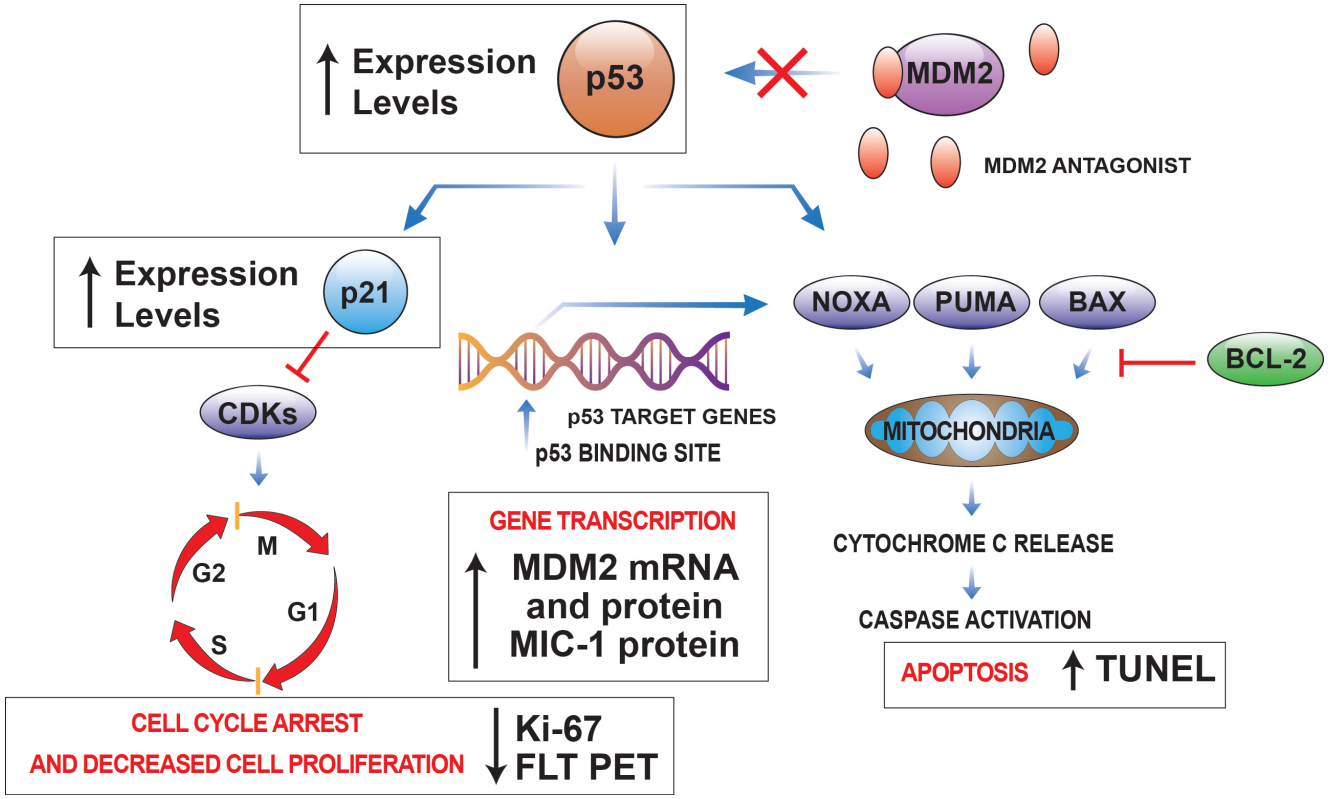
In regards to the first goal, clinical activity of MDM2 antagonism has been confirmed in adult patients with AML and solid tumors. Tovar et al. showed proof-of-concept that RO5045337 activates p53 and promotes cell cycle arrest and apoptosis in vitro, as well as tumor inhibition and regression in vivo (Tovar et al. 2013). Correspondingly, downstream biomarkers of p53 activation were analyzed in clinical studies of MDM2 antagonists to correlate tumor response outcomes with these indicators. In a Phase I study of RO5045337 in patients with MDM2-amplified, well-differentiated or dedifferentiated liposarcomas, target genes of p53 transactivation (i.e., *MDM2*), proteins downstream of activated p53 signaling (i.e., p21, Ki-67, MIC-1), and apoptosis have been analyzed (Ray-Coquard et al. 2012). Changes in each of these biomarkers were consistent with the expected mechanism of action of MDM2 antagonists (Figure 7) although changes in p53 and p21 concentration, *MDM2* mRNA expression, and the number of Ki-67 positive cells did not significantly correlate with drug exposure, but may have been limited by the small number of patients studied. In contrast, MIC-1 was significantly correlated with drug exposure and hematological activity. Preliminary evidence from a Phase I study of RO5503781 suggests that MIC-1 data trended toward greater p53 activation with a daily dosing schedule (Siu et al. 2014). These data suggest that MIC-1 concentrations could function as a pharmacodynamic biomarker to determine p53 reactivation following MDM2 antagonism treatment (Ray-Coquard et al. 2012). The advantage to using MIC-1 as a pharmacodynamic biomarker is that it can be measured in serum without obtaining tumor samples for biopsy (Yang et al. 2003).

With regard to the second biomarker development goal, clinical studies have suggested that *TP53* mutational status alone is insufficient to discriminate between likely responders and non-responders to RO5503781 treatment. As discussed, nonclinical evidence demonstrates that RO5503781 activates the p53 pathway and induces cell cycle arrest and/or apoptosis in a variety of tumor types that express wild-type *TP53*. While a biological rationale for the potential lack of efficacy of an MDM2 antagonist in patients harboring p53 mutations exists, not all *TP53* mutations cause a loss of p53 tumor suppressor activity. Many mutations in the *TP53* promoter region cause

epigenetic changes resulting in low levels of mRNA production in patients with ALL (Saldana-Meyer and Recillas-Targa 2011; Agirre et al. 2003). Clinical studies of RO5503781 and first-generation MDM2 antagonist RO5045337 have demonstrated evidence of clinical activity in patients with *TP53* mutations in the protein-coding region, including the achievement of CR in a patient with AML. Retention of p53 function may be a more important predictor of response to RO5503781 treatment than *TP53* mutational status.

Development of predictive markers of RO5503781 treatment outcomes would offer the potential to target pediatric patients who would derive the most benefit for therapy. Currently there are no validated biomarkers which robustly predict response to RO5503781 with a sensitivity and specificity profile for suitable inclusion as a pre-selection strategy. While *TP53* mutations tend to associate with non-responder patients, evidence of clinical activity for MDM2 antagonists in patients harboring *TP53* alterations complicates such an adoptive strategy for patient selection. Thus, its inclusion as a pediatric pre-selection biomarker is unsupported at present. Further, Roche previously discussed a gene signature that displays significant and promising association with clinical response in retrospective analyses (Zhong et al., unpublished data); however, such multianalyte tests rely on continuous variable measurements, and cut-points remain undefined as do sensitivity and specificity profiles for response discrimination. Roche remains optimistic that information gained from continuing assessments in ongoing adult studies will provide an evolving level of evidence that may be utilized in the future to provide criteria for selection of patients with the highest chance of response.

Figure 7 Core PD/Biomarkers of p53 Activation Assessed in the Clinic



BAX = BCL-2-associated X protein; BCL-2 = B cell lymphoma 2 protein; CDKs = cyclin-dependent kinases; FLT PET = 18F-3'-fluoro-3-deoxy-L-thymidine positron emission tomography; G1 = Growth 1/Gap 1 phase; G2 = Growth 2/Gap 2 phase; M = mitosis; MDM2 = mouse double minute 2; MIC-1 = macrophage inhibitory cytokine 1; NOXA = phorbol-12-myristate-13-acetate-induced protein 1, p21 = cyclin-dependent kinase inhibitor 1A (CDKN1A, CIP1); PUMA = p53 upregulated modulator of apoptosis; S = synthesis phase; TUNEL = Terminal deoxynucleotidyl transferase dUTP nick end labeling.

7.7.2 Pharmacokinetic Variability

Clinical studies in adults with RO5503781 have shown that there is substantial variability in exposure between patients. Given the on-target exposure-related toxicity of p53-induced myelosuppression, potential clinical benefits and the ability to combine with cytotoxic therapies may be hampered by a narrow therapeutic window. Thus, pediatric PK evaluation will be a critical factor for safety and successful pediatric development.

PK assessment is one of the key outcomes for the planned pediatric studies. RO5503781 is primarily metabolized in the liver. Because enzyme expression may change in pediatric patients as they develop, PK analysis will be conducted in all patients enrolled in the planned Phase I study to provide information on RO5503781 clearance. Likewise, for safety, variability of exposure in children will be carefully examined. For the initial planned pediatric study, pediatric PK profiles covering a range of ages and body weights will be projected to support the starting dose selected for children. One or more methods (e.g., physiologically-based PK, scaling of clearance and volume of distribution at steady state, accounting for the inter-patient variability observed in adults, etc.) may be used to project the PK for children at the starting dose.

7.7.3 Formulation

The adult solid dosage form is a film-coated tablet. In order to overcome bioavailability limitations, the selected oral solid dosage formulation contains the active pharmaceutical ingredient as a solid dispersion in amorphous form. For the proposed pediatric study for children $\geq$2 years of age who are able to swallow, smaller, film-coated tablets were developed. The practical limitation of the development of a pediatric oral solid dosage form is the relationship between necessary dose, the maximal drug load that is possible in a child–appropriate size tablet, and the number of tablets. As an example, the currently available 20 mg film-coated tablet, which is the smallest possible dose-proportional tablet, is 5 mm in diameter.

The ability of children to swallow tablets is not strictly a function of age, and therefore there will be children less than 6 years of age who can participate in the trial based on their relative maturity in this regard. Conversely, children over 6 years of age may not be able to tolerate swallowing tablets. Roche is exploring the development of an age-appropriate formulation for those children, both under 2 years of age, and older, who have difficulty in swallowing tablets. However, the poor solubility of RO5503781 and the sensitivity of its amorphous state to moisture strongly limit the possibility of developing a liquid formulation. To address this challenge, Roche is planning to develop mini-tablets and an ad hoc suspension formulation. Roche proposes to integrate the use of both tablets and age-appropriate oral formulations into the planned pediatric studies in order to include children of all age groups as early as possible.

As an alternative strategy with potential as an age appropriate-formulation, Roche is concurrently developing an intravenously administered pegylated prodrug of RO5503781 as a unique new molecular entity. An intravenous MDM2 antagonist has been developed in order to decrease the variability in exposure seen with the oral compound, RO5503781, and to allow expansion into indications (such as pediatrics) where patients cannot swallow or absorb the oral compound. It is important to note that the nonclinical data for the intravenous formulation is consistent with that of the oral formulation. A Phase I entry-into-human study has recently been initiated in adult patients with solid tumors and AML.

8. EUROPEAN PAEDIATRIC INVESTIGATION PLAN AND ANY ONGOING CLINICAL TRIALS IN PEDIATRICS

In fulfillment of the requirements as set by Article 7 of the Pediatric Regulation in Europe, a PIP procedure commenced on the 12 September 2013. The PDCO adopted an opinion agreeing to the PIP on 12 September 2014 for the treatment of AML, ALL, and malignant neoplasms (except nervous system, hematopoietic and lymphoid tissue).

The development of an age-appropriate, liquid or solid dosage form for oral use as well as the proposed nonclinical study and clinical studies in pediatrics as mentioned in Section 7 are reflective of the content of the PIP; modifications to be expected.

Currently there are no RO5503781 pediatric clinical trials ongoing.

9. CONCLUSIONS

The unique mechanism of action and evidence of nonclinical efficacy provides strong scientific support for the development of RO5503781 in pediatric cancers with functional p53 tumors. In adults, RO5503781 has shown promising efficacy and acceptable safety in patients with AML. The pediatric development plan for RO5503781 will take into consideration each of these elements. Roche welcomes discussion with the Pediatric Oncology Subcommittee of the ODAC regarding the future development of RO5503781 in pediatric patients.

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